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Cloning and tissue distribution of the human G protein β5 cDNA

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Abstract

Heterotrimeric G proteins integrate signals between receptors and effector proteins. We have cloned the human β5 subunit from a human brain cDNA library. The clone has a 1059 bp open reading frame and is highly homologous to the murine clone. In contrast to the brain specific mouse β5, northern analysis showed it to be expressed in multiple tissues.

Keywords: Tissue distribution; G protein; β Subunit; cDNA; Gene expression

The intracellular responses to stimuli such as hormones, neurotransmitters and light are mediated through integral membrane receptor proteins which couple to effector systems through heterotrimeric guanine nucleotide binding proteins (G proteins). These G proteins consist of α, β and γ subunits and multiple isoforms of each subunit exist. To date, at least 17 α subunit genes which through alternative splicing give rise to 21 different proteins, five β subunits and 11 γ subunits have been identified [1–3]. Upon activation, the G protein α subunit dissociates from the βγ dimer, both components can influence the activity of effector proteins such as phospholipase C, adenylate cyclase, cGMP phosphodiesterase and various ion channels, thus allowing receptors to couple to bifurcating signalling pathways.

The G protein β subunits are highly homologous and form a stable seven-bladed propeller structure, interacting with the γ subunits through their N terminus [4–6]. The β and γ subunits are found as a stable dimer in vivo and the potential for 55 different dimers allows for much diversity. β5 is the most structurally diverse of the β subunits sharing only 50% homology with the other four subtypes, this contrasts with the 83% minimum homology found between β1–β4. Interestingly, a novel form of Gβ5 has recently been identified in the mouse retina [7]. This subunit termed Gβ5L is identical to Gβ5 but has an additional 126 bp of coding region at the 5′ arising from an additional exon. The discovery of this novel retinal isoform suggests an interesting second line of diversity in G protein signalling.

Here we report the cloning and tissue distribution of the human G protein β5 subunit. This human subunit is highly homologous to the mouse β5 subunit which is predominantly expressed in the brain. However, the hGβ5 is expressed in other tissues such as the heart in addition to high levels throughout the brain.

The hGβ5 cDNA was cloned from a human brain lambda gt10 cDNA library (Clontech, Palo Alto, CA)

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using a degenerate PCR approach. The degenerate primers were based on the mouse $\beta 5$ sequence (Forward GGNCA$^{A}/_{G}$TT$^{T}/_{C}$GTNATGGAAA, reverse $^{A}/_{G}$A$^{A}/_{G}$AANGCNGTNCC$^{A}/_{G}$TC) and were used to clone a 878 base pair partial coding sequence using the following PCR protocol on a Perkin-Elmer 9600 thermocycler: denature 95°C 5 min, followed by 35 cycles of denature 95°C 30 s, anneal 1 min 50°C, extension 1 min 72°C, the reaction was terminated with a final extension at 72°C for 5 min. The resulting band was purified, ligated into the cloning vector pT7Blue (Novagen, Madison, WI) and was sequenced using an ABI 373 automated sequencer. Full length clones were obtained using a nested PCR RACE approach. In view of the non-orientated inserts in λgt10 libraries, duplicate reactions were performed using either the λgt10 forward primer AGCAAGTTCAGCCTGGTTAAG or λgt10 reverse primer TTATGAGTATTTCTTCCAGGG (Clontech) with specific hG$\beta 5$ primers. For the 5′ cloning the first round of PCR utilised the hG$\beta 5$ reverse primer CACCTTCCCATCCTGTGACG with the λgt10 primers. A total of 5 μl of the product reaction was subject to a second round of PCR using a primer upstream of the previous one CTTTGCACCAGTCCATGCAC. The 3′ was similarly cloned using the primer CTGGGATGTTCTCAAAGGG with the λgt10 primers followed by a second round with CCGCGTTAGCACTCTACGAG. These bands were isolated and sequenced as previously described. PCR was then used to obtain a full length clone.

The tissue distribution of hG$\beta 5$ was investigated on both human and mouse multi tissue (Clontech) and human-multi brain region Northern blots (Clontech). The blots were pre-hybridised with ExpressHyb solution (Clontech) at 65°C and hybridised with a ^{32}P labelled probe prepared by random hexamer labelling of the 878 partial coding region of hG$\beta 5$ (bases 145–1023). The human blot was then washed to a stringency of 0.1 × SSC 0.1% SDS at 65°C and exposed for 48 h using a Molecular Dynamics phosphor screen. Due to the lower homology of the probe with the mouse mRNA, the mouse blot was washed 0.1 × SSC 0.1% SDS at 50°C. The probe was shown to be specific for $\beta 5$ under both conditions (data not shown).

Fig. 1 shows the nucleotide sequence and predicted protein sequence of the clone. There is a 1059

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 TCCAAGCTGAATTCCGGGGACGGCTGCTGGAGCGGCGCCCGCCGCGGCT -51
CAGCGCATTCCCGCTCTCCGCTTCCCTCTCCGCTGCGTCCCCGCGCGAAG  -1
ATGGCAACCGAGGGGCTGCACGAGAACGAGACGCTGGCGTCGCTGAAGAG  50
 M  A  T  E  G  L  H  E  N  E  T  L  A  S  L  K  S
CGAGGCCGAGAGCCTCAAGGGCAAGCTGGAGGAGGAGCGAGCCAAGCTGC 100
  E  A  E  S  L  K  G  K  L  E  E  E  R  A  K  L
ACGATGTGGAGCTGCACCAGGTGGCGGAGCGGGTGGAGGCCCTGGGGCAG 150
H  D  V  E  L  H  Q  V  A  E  R  V  E  A  L  G  Q
TTTGTCATGAAGACCAGAAGGACCCTCAAAGGCCACGGGAACAAAGTCCT 200
 F  V  M  K  T  R  R  T  L  K  G  H  G  N  K  V  L
GTGCATGGACTGGTGCAAAGATAAGAGGAGGATCGTGAGCTCGTCACAGG 250
  C  M  D  W  C  K  D  K  R  R  I  V  S  S  S  Q
ATGGGAAGGTGATCGTGTGGGATTCCTTCACCACAAACAAGGAGCACGCG 300
D  G  K  V  I  V  W  D  S  F  T  T  N  K  E  H  A
GTCACCATGCCCTGCACGTGGGTGATGGCATGTGCTTATGCCCCATCGGG 350
 V  T  M  P  C  T  W  V  M  A  C  A  Y  A  P  S  G
ATGTGCCATTGCTTGTGGTGGTTTGGATAATAAGTGTTCTGTGTACCCCT 400
  C  A  I  A  C  G  G  L  D  N  K  C  S  V  Y  P
TGACGTTTGACAAAAATGAAAACATGGCTGCCAAAAAGAAGTCTGTTGCT 450
L  T  F  D  K  N  E  N  M  A  A  K  K  K  S  V  A
ATGCACACCAACTACCTGTCGGCCTGCAGCTTCACCAACTCTGACATGCA 500
 M  H  T  N  Y  L  S  A  C  S  F  T  N  S  D  M  Q
GATCCTGACAGCGAGCGGCGATGGCACATGTGCCCTGTGGGACGTGGAGA 550
  I  L  T  A  S  G  D  G  T  C  A  L  W  D  V  E
GCGGGCAGCTGCTGCAGAGCTTCCACGGACATGGGGCTGACGTCCTCTGC 600
S  G  Q  L  L  Q  S  F  H  G  H  G  A  D  V  L  C
TTGGACCTGGCCCCCTCAGAAACTGGAAACACCTTCGTGTCTGGGGGATG 650
 L  D  L  A  P  S  E  T  G  N  T  F  V  S  G  G  C
TGACAAGAAAGCCATGGTGTGGGACATGCGCTCCGGCCAGTGCGTGCAGG 700
  D  K  K  A  M  V  W  D  M  R  S  G  Q  C  V  Q
CCTTTGAAACACATGAATCCGACATCAACAGTGTCCGGTACTACCCCAGT 750
A  F  E  T  H  E  S  D  I  N  S  V  R  Y  Y  P  S
GGAGATGCCTTTGCTTCAGGGTCAGATGACGCTACGTGTCGCCTCTATGA 800
 G  D  A  F  A  S  G  S  D  D  A  T  C  R  L  Y  D
CCTGCGGGCAGATAGGGAGGTTGCCATCTATTCCAAAGAAAGCATCATAT 850
  L  R  A  D  R  E  V  A  I  Y  S  K  E  S  I  I
TTGGAGCATCCAGCGTGGACTTCTCCCTCAGTGGTCGCCTGCTGTTTGCT 900
F  G  A  S  S  V  D  F  S  L  S  G  R  L  L  F  A
GGATACAATGATTACACTATCAACGTCTGGGATGTTCTCAAAGGGTCCCG 950
 G  Y  N  D  Y  T  I  N  V  W  D  V  L  K  G  S  R
GGTCTCCATCCTGTTTGGACATGAAAACCGCGTTAGCACTCTACGAGTTT 1000
  V  S  I  L  F  G  H  E  N  R  V  S  T  L  R  V
CCCCCGATGGGACTGCTTTCTGCTCTGGATCATGGGATCATACCCTCAGA 1050
S  P  D  G  T  A  F  C  S  G  S  W  D  H  T  L  R
GTCTGGGCCTAATCATCTTCTGACAGTGCACTCATGTATACCTGAGAATT 1200
 V  W  A
TGAAATCTTCACATGTAAATAGATATTACTTCTAGAGGAGCAGAGTTTAT
TGCAGTGTAGCTTAGGGGAGCAACCCATGGCTCACAGGTCACTAAGCGTC
TCCAATATGACTATTAAAACTGTCACCTCTGGAAATACACTAGTGTGAGC
CTTCAGCACTGCGAGAATACCTTCAAGTACAGTATTTTTCTTTTGGAACA
CTTTTTAAAATGTATCTGTTTTTAAGGTTATTCTAAATTATAGTAGCCTC
AACTCATTCTGTCACCAGTAGAATTCTTTTGCTTTTTACCCTGGAAGAAA
TACTCATAAG                                        1511
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Fig. 1. DNA and predicted amino acid sequence of the hG$\beta 5$. The nucleotide numbering is indicated and positive numbering starts at the initiation codon ATG. The probe used in Fig. 3 corresponds to bases 145–1023.

base pair open reading frame encoding a 353 amino acid protein of estimated molecular weight 38.7 kDa. The human sequence is highly conserved with the mouse sequence showing 90.7% DNA homology and 99.4% homology at the protein level (351 amino acids out of the 353 are conserved). The amino acid changes E4 (D in mouse) and I 242 (V in mouse) are conservative changes and are thought to represent bonafide substitutions since alignments of several ESTs confirm these changes (Fig. 2). This high conservation across species is common for the β subunits, the bovine and human homologues of $\beta 1$, $\beta 2$

A.

```
hGβ5       ATGGCAACCGAGGGGCTGCACGAGAACGAGACGCTGGCGT 40
est19480   ATGGCAACCGAGG
             M  A  T  E  G  L  H  E  N  E  T  L  A  13
mGβ5                D
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B.

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hGβ5       CTTTGAAACACATGAATCTGACATCAACAGTGTCCGGTAC  741
W25896     CTTTGAAACACATGAATCTGACATCAACAGTGTCCGGTAC
AA158679   CTTTGAAACACATGAATCTGACATCAACAGTGTCCGGTAC
             F  E  T  H  E  S  D  I  N  S  V  R  Y  247
mGβ5                                  V
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Fig. 2. Alignment of regions hGβ5 and several ESTs confirming the amino acid changes in the human sequence (A) E4, (B) I242. EST 19480 was taken from Ref. [7]. W25896 and AA158679 were found by searching dbEST with the hGβ5 sequence.

and β3 are 100% conserved at the protein level and 90% homologous at the nucleotide level.

hGβ5 RNA was detected in poly A + RNA extracted from all tissues tested. Three bands were detected, the major band being approximately 3.0 kb, two minors ones were seen at approximately 2.0 kb and 9.0 kb. Although this study employed a coding region probe, under the stringent conditions used, the probe was shown to be specific for β5 (data not shown). The relative intensities of the three bands were the same in all regions studied, the expression levels are whole brain > pancreas > heart = kidney > placenta > liver > lung = skeletal muscle (Fig. 3a). The higher expression in heart, kidney and pancreas is very different from that seen in mouse which is highly enriched in the brain (Fig. 3b) although message was detectable in all tissues tested. These data would support the results of Watson et al. [2] who described a very selective distribution. Trace amounts in a range of tissues were reported and the greater sensitivity of the phosphorimager used in the present study may account for the seemingly higher expression levels reported here.

Within the brain, hGβ5 RNA was detected at some level in all regions studied (Fig. 4), with the highest levels of expression being detected in the cerebellum, cerebral cortex, occipital pole, frontal lobe, temporal lobe and caudate putamen. The lowest expression was seen in non-neuronal and non-central tissue (corpus callosum and spinal cord). The higher expression in neuronal tissue highlights its role in brain signalling. The distribution described here is in general agreement with an in situ hybridisation study

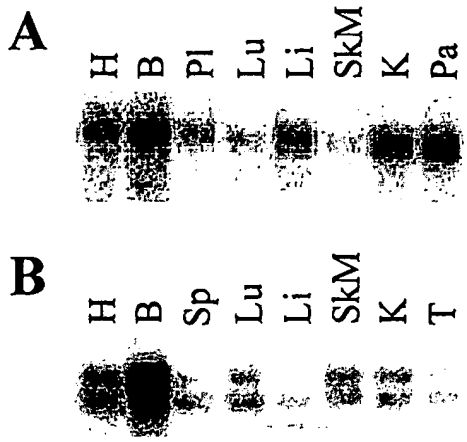

Fig. 3. Northern analysis of human (a) and mouse (b) Gβ5 expression. Each lane contains approximately 2 μg poly A+ RNA. The blot was prehybridised with ExpressHyb solution (Clontech) and hybridised with a ^{32}P labelled 879 bp coding region probe, then washed to a stringency of 0.1× SSC 0.1% SDS at 65°C for the human blot and 0.1× SSC 0.1% SDS at 50°C for the mouse. The blot was exposed to a phosphorimager screen for 48 h. The major 3.0 kb band is shown. H = heart; B = brain; Pl = placenta; Lu = lung; Li = liver; SkM = skeletal muscle; K = kidney; Pa = pancreas; Sp = spleen; T = testis.

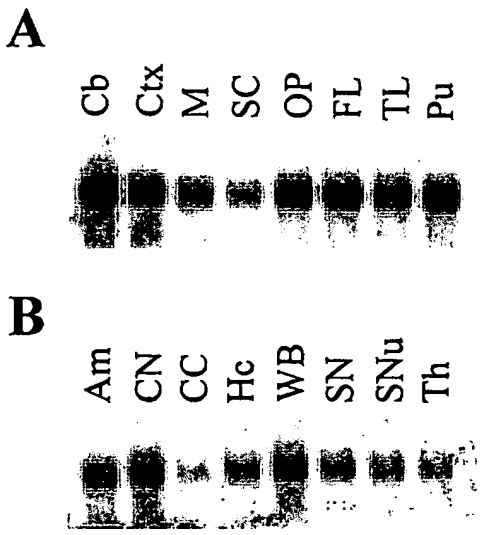

Fig. 4. Expression of Gβ5 in human brain. A human multi brain northern blot was hybridised with a ^{32}P labelled 879 bp coding region probe, then washed to a stringency of 0.1× SSC 0.1% SDS at 65°C and exposed on a phosphoimager screen for 48 h. Cb = cerebellum; Ctx = cerebral cortex; M = medulla; SC = spinal cord; OP = occipital pole; FL = frontal lobe; TL = temporal lobe; Pu = caudate putamen. Am = Amygdala; CN = Caudate nucleus; CC = Corpus Callosum; Hc = Hippocampus; WB = whole brain; SN = substantia nigra; SNu = subthalamic nucleus; Th = thalamus.

[8] although this study would indicate a higher level of hippocampal expression.

In summary, we have cloned the hGβ5 subunit from a λgt10 brain library. The human form has high homology with the murine clone, differing by just two amino, although the pattern of expression of hGβ5 is more widespread than the mouse. This clone will help elucidate species and subtype specific details of G protein signalling.

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